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Catalysis: transition-state molecular recognition?

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Commentary

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Abstract

The key to understanding the fundamental processes of catalysis is the transition state (TS): indeed, catalysis is a transition-state molecular recognition event. Practical objectives, such as the design of TS analogues as potential drugs, or the design of synthetic catalysts (including catalytic antibodies), require prior knowledge of the TS structure to be mimicked. Examples, both old and new, of computational modelling studies are discussed, which illustrate this fundamental concept. It is shown that reactant binding is intrinsically inhibitory, and that attempts to design catalysts that focus simply upon attractive interactions in a binding site may fail. Free-energy changes along the reaction coordinate for S_N2 methyl transfer catalysed by the enzyme catechol-*O*-methyl transferase are described and compared with those for a model reaction in water, as computed by hybrid quantum-mechanical/molecular-mechanical molecular dynamics simulations. The case is discussed of molecular recognition in a xylanase enzyme that stabilises its sugar substrate in a (normally unfavourable) boat conformation and in which a single-atom mutation affects the free-energy of activation dramatically.

Introduction

“Molecular recognition of transition states” was the title of a paper presented by Kirby [1] at a discussion held in April 1993 on the chemistry of biological molecular recognition; he addressed the fundamental question of how enzymes lower the free energies of the transition states for the reactions they catalyse, with reference to his own elegant experimental studies on catalysis. In March 1991, at a workshop held under the auspices of the Science and Engineering Research Council’s Molecular Recognition Initiative, I presented a paper on theo-

retical modelling of transition states for biochemical processes, which included a computational model for carbonyl reduction catalysed by lactate dehydrogenase [2]. The abstract for this workshop presentation began with the following sentence: The key to understanding of the fundamental processes of catalysis is the transition state; indeed, “catalysis is a transition-state molecular recognition event”. The present paper discusses cases of methyl transfer and of glycoside hydrolysis to illustrate and to update the same theme from a computational point of view.

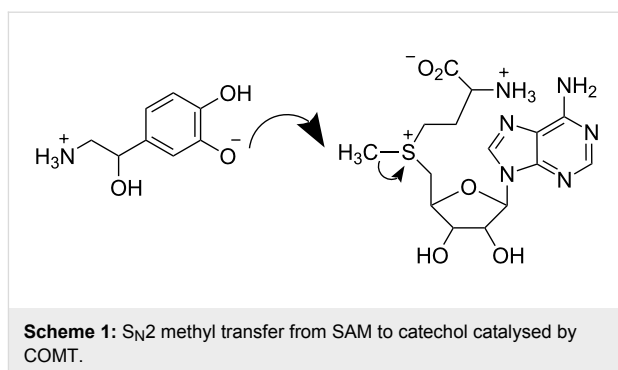
fully with the substrate in combining with the enzyme, which in this respect would be similar in behaviour to antibodies; but an enzyme complementary to a strained substrate molecule would attract more strongly to itself a molecule resembling the strained substrate molecule than it would the substrate molecule.” This clearly implies a consideration of the relative extent of binding interactions of the reactant state and TS with an enzyme, and of the inhibitory nature of the former.

The essential importance of *preferential* TS stabilisation was absolutely explicit in Schowen’s treatment [6]: “A complete understanding of enzyme catalysis ... resolves into a characterisation of two binding processes: that for the transition state, which yields a model for catalysis, and that for the reactant state, which yields a model for ... inhibitory effects ... The differential stabilisation of the transition state (total stabilisation of the transition state minus stabilisation of reactant species) always gives the catalytic acceleration.” Recently, Simón and Goodman [10] have astutely observed that an optimal catalyst does not simply maximise TS stabilisation per se, but rather achieves a maximal reduction in barrier height by means of differential stabilisation. The cases discussed below all exemplify TS molecular recognition and stabilisation *relative* to the reactant state.

Catalyst design: preferential TS binding

Methyl group transfer from an electrophile to a nucleophile by an S_N2 mechanism is an archetypal reaction in organic chemistry and an important process in biochemistry. Catechol-*O*-methyl transferase (COMT) catalyses methyl transfer from *S*-adenosylmethionine (SAM) to a catechol (Scheme 1), and this reaction manifests an unusually large inverse secondary kinetic isotope effect as compared with a model, uncatalysed reaction in solution: the isotope effect $k^{CH_3}/k^{CD_3} = 0.83 \pm 0.05$ for methylation of 3,4-dihydroxyacetophenone with SAM at 37 °C catalysed by COMT was found [11] to be more inverse than the value of $k_{CH_3}/k_{CD_3} = 0.97 \pm 0.02$ for methylation of methoxide ion by *S*-methylidibenzothiophenium ion at 25 °C in methanol [12]. According to the orthodox view, Schowen and co-workers interpreted these observations in terms of a tighter S_N2 transition state for the COMT-catalysed reaction than for the non-enzymic reaction, and consequently proposed the “compression hypothesis” for enzymic methyl transfer as a possible explanation [13].

As outlined above, the power of any catalyst derives fundamentally from its ability to stabilise the TS relative to the reactant state, as compared with the uncatalysed reaction. This requires effective discrimination between the reactant state and the TS. In the case of methyl transfer, stabilising enzyme-substrate interactions (① in Figure 2) probably do not provide any



significant degree of discrimination, since the geometrical and electronic changes occurring do not provide sufficient differences; thus

$$\left| \Delta E^R_{\text{stabilise}} \right| \approx \left| \Delta E^T_{\text{stabilise}} \right| \quad (1)$$

The key proposal of the compression hypothesis is the following: if the TS for S_N2 methyl transfer is more plastic than the reactant state for the catalysed process, then mechanical compression by the enzyme (② in Figure 2) might destabilise the reactants more than the TS. In other words, the energetic penalty for deforming the structure to a given degree is greater for the reactant state than for the TS:

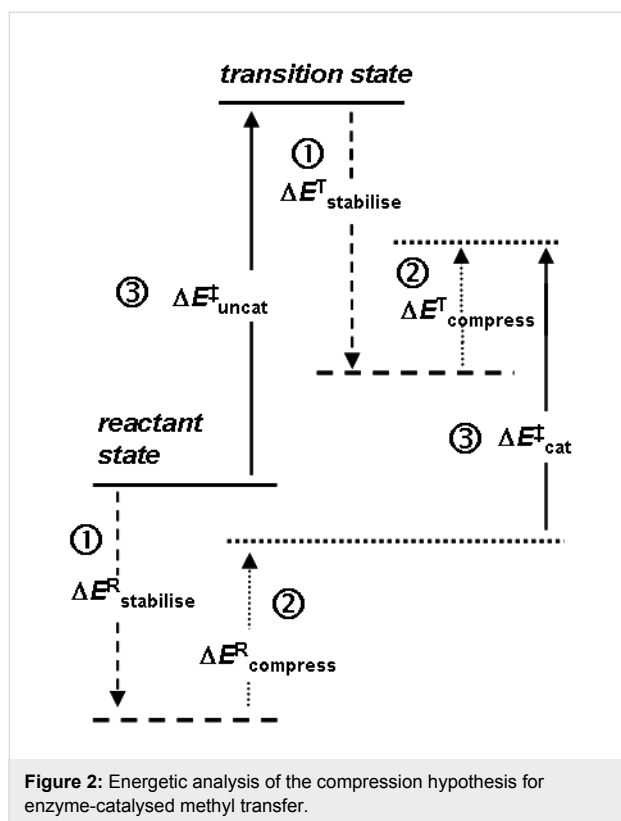
$$\left| \Delta E^R_{\text{compress}} \right| > \left| \Delta E^T_{\text{compress}} \right| \quad (2)$$

The net effect (③ in Figure 2) is the reduction of the barrier for the catalysed reaction as compared with that for the uncatalysed process:

$$\Delta E^\ddagger_{\text{cat}} < \Delta E^\ddagger_{\text{uncat}} \quad (3)$$

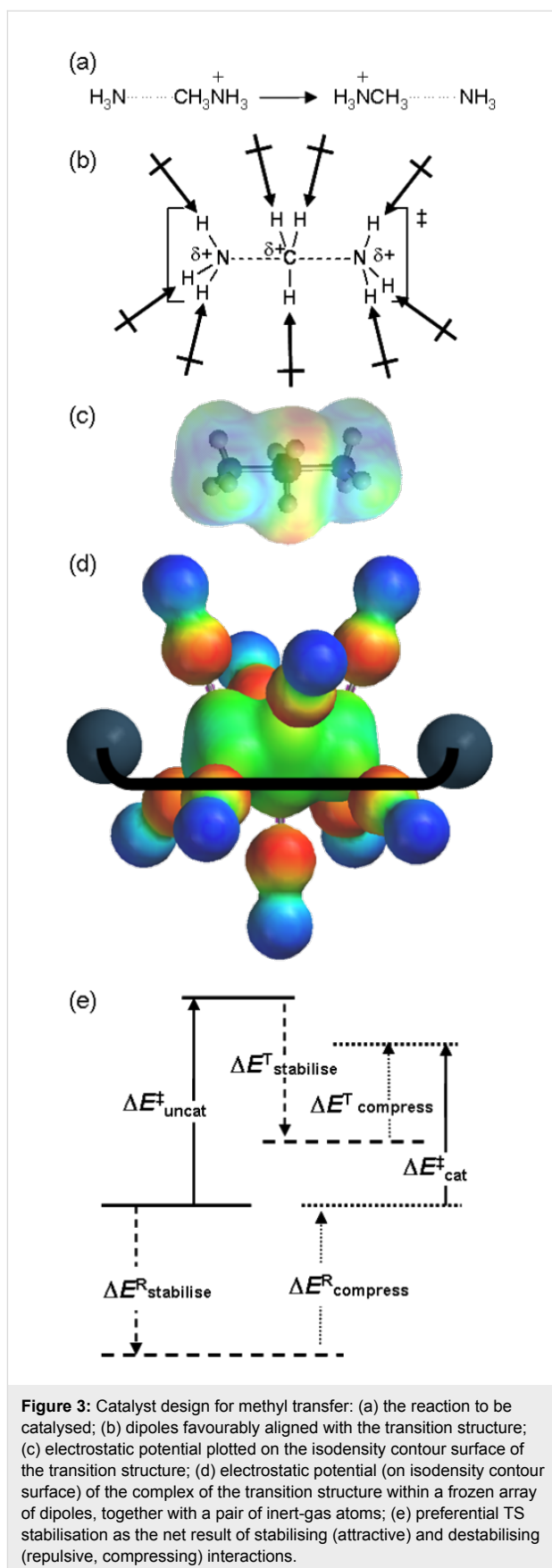
As a consequence of (intrinsically unfavourable) compression of the S_N2 TS in the enzymic reaction, the enzyme is able to distinguish the TS structurally from the preceding reactant state and the succeeding product state in order to stabilise the TS specifically. Thus, compression may serve to achieve efficient catalysis, with a large V_{max} at the expense of a slight reduction in V_{max}/K_m . The importance for enzyme catalysis of destabilisation as well as binding has also been noted by Jencks [14].

Some years ago I performed an ab initio Hartree–Fock investigation [15], intended to test the validity of the compression hypothesis; this exercise amounted to the computational design of a catalyst for the identity S_N2 methyl transfer from methylammonium to ammonia (Figure 3a). The transition structure for



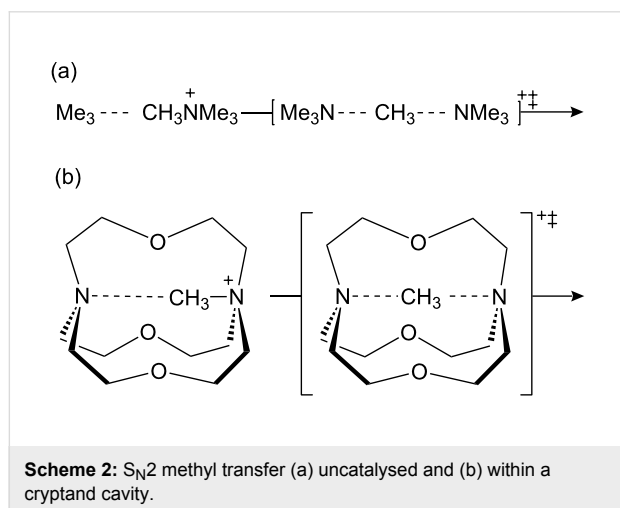
this reaction has an overall positive charge, and a reasonable strategy for its stabilisation seemed to be to construct an array of point charges, such that each N–H or C–H bond was perfectly aligned with the negative end of a dipole (Figure 3b). However, when both the transition structure and the ion-molecule reactant complex were reoptimised within the frozen array of point charges, it transpired that the stabilisation energy $\Delta E^R_{\text{stabilise}}$ of the latter was greater than the stabilisation energy $\Delta E^T_{\text{stabilise}}$ of the former (Figure 3e). Unintentionally, the barrier for S_N2 methyl transfer with the “catalyst” was higher than that without: inhibition, or anti-catalysis, had been achieved. With hindsight, it may be seen from the electrostatic potential of the transition structure (represented by colour on an electron density contour in Figure 3c), that the transferring methyl group is unlikely to interact favourably with the dipoles intended to do so: the electrostatic potential for the transition structure within the catalyst (Figure 3d) appears uniform. The catalyst dipoles interact more strongly with the localised charge on the reactant (or product) ion-molecule complex than with the delocalised charges on the atoms of the transition structure.

However, when a pair of inert-gas atoms (grey spheres in Figure 3d) was placed on the N \cdots C \cdots N axis so as to impose repulsive interactions on both the reactant and transition structures sandwiched between them, the destabilising effect $\Delta E^R_{\text{compress}}$ on the former could be adjusted (by appropriate



choice of the fixed separation of the inert-gas atoms) to be significantly larger than the destabilising effect $\Delta E^{\text{T}}_{\text{compress}}$ on the latter. The net effect of the attractive and repulsive components of the catalyst yielded $\Delta E^{\ddagger}_{\text{cat}} < \Delta E^{\ddagger}_{\text{uncat}}$ (Figure 3e), because the preferential destabilisation of the reactant state by compression outweighed its preferential stabilisation by attractive interactions with the dipole array; alternatively, $\Delta E^{\text{R}}_{\text{bind}} (= \Delta E^{\text{R}}_{\text{stabilise}} + \Delta E^{\text{R}}_{\text{compress}}) < \Delta E^{\text{T}}_{\text{bind}} (= \Delta E^{\text{T}}_{\text{stabilise}} + \Delta E^{\text{T}}_{\text{compress}})$ leading to net TS stabilisation.

Later we proposed [16] a more realistic catalyst design for methyl transfer in the shape of inside-methylated [1.1.1]cryptand (Scheme 2). B3LYP/6-31G* calculations predicted the inter-bridgehead N...N distance in cryptand (b) to be 0.75 Å shorter than in the ion-molecule complex between trimethylamine and tetramethylammonium (a), indicating compression along the N...C...N axis, but more significantly the corresponding difference in the corresponding transition structures was only 0.35 Å. In other words, the change from reactant complex to transition structure was 0.4 Å less for the compressed reaction (b) than for the uncompressed reaction (a); moreover, the potential energy barrier for (b) was 22 kJ mol^{−1} less than for (a), and the α -D₃ KIEs were more inverse (0.91 vs 0.93) for (b) than for (a). These results were consistent with the compression hypothesis for catalysis of methyl transfer.



Origin of COMT catalytic power

To assess whether compression actually operates in COMT-catalysed methyl transfer, hybrid QM/MM calculations have been performed at the AM1/MM level [17–19]. The secondary α -D₃ KIE for the COMT-catalysed reaction (Scheme 1) was calculated to be more inverse than for the same reaction in water [18], but this preliminary result was based upon single structures for the reactant complex and transition state of the enzymic and non-enzymic reactions. Recently we performed

extensive AM1/OPLS/TIP3P simulations [19] with ensemble averaging to include the effect of thermal fluctuations in the enzyme and solvent environments to obtain a value for the α -D₃ KIE = 0.82 ± 0.05 , which is in excellent accord with the experimental value [11] of $V^{\text{CH}_3}/V^{\text{CD}_3} = 0.83 \pm 0.05$ for methylation of 3,4-dihydroxyacetophenone with SAM at 37 °C catalysed by COMT. In contrast, we calculated $k_{\text{CH}_3}/k_{\text{CD}_3} = 0.99 \pm 0.16$ for methylation of methoxide ion by *S*-methylidibenzothiophenium ion at 25 °C in methanol, as compared with the experimental value [12] of 0.97 ± 0.02 . The computational results reproduce the experimental observation of a significantly more inverse value of α -D₃ KIE for enzyme-catalysed than for uncatalysed methyl transfer in solution. However, the average values for the making and breaking bonds between C_α and, respectively, the nucleophile and nucleofuge in the nearly collinear TS for the COMT-catalysed reaction were computed as 2.06 ± 0.02 Å and 2.11 ± 0.01 Å, the sum of which is scarcely different from the sum of the corresponding average bond lengths, 2.18 ± 0.04 Å and 2.00 ± 0.04 Å, for the uncatalysed reaction. Thus the simulations did not provide any structural evidence for compression.

It is instructive to analyse the various energetic contributions to catalysis (Figure 4) by COMT by means of appropriate computer simulations, as was done in an earlier study [17]. (N.B. The terminology and notation employed here differ from that work.) The potential of mean force (PMF), computed from MD simulations at the AM1/CHARMM/TIP3P level with umbrella sampling along a reaction coordinate defined as the difference in bond lengths from C_α to the nucleophile and nucleofuge, predicted a 44 kJ mol^{−1} increase $\Delta G^{\ddagger}_{\text{enz}}$ in free energy in going from the enzymic reactant complex ES^R_{enz} to the enzymic transition state ES^T_{enz} for the COMT-catalysed reaction at 300 K. An analogous PMF for exactly the same reaction occurring in water without COMT yielded a free energy minimum for a solvent-separated ion-pair reactant complex S^R_{aq}; if this species were taken as the reference state for both catalysed and uncatalysed reactions, the reduction in barrier height would simply be equal to $\Delta G^{\text{T}}_{\text{bind}}$, the TS stabilisation. In the published analysis [17], the free energy barrier $\Delta G^{\ddagger}_{\text{aq}} = 82$ kJ mol^{−1} for the uncatalysed reaction in aqueous solution was considered as the sum of two terms: (i) a distortion energy $\Delta G^{\text{R}}_{\text{dist}} = 30$ kJ mol^{−1} for going from S^R_{aq} to a contact ion-pair S^R_{enz} in solution having the same geometry as that of the substrate-derived part of the enzymic reactant complex ES^R_{enz} and (ii) an activation free energy $\Delta G^{\text{R}}_{\text{act}} = 52$ kJ mol^{−1} to the transition state S^T_{aq}. The sum of $\Delta G^{\text{R}}_{\text{dist}}$ and the interaction energy $\Delta G^{\text{R}}_{\text{int}}$ is equal to the apparent binding energy $\Delta G^{\text{R}}_{\text{bind}}$. The magnitude of the enzyme catalytic power $\Delta G^{\ddagger}_{\text{aq}} - \Delta G^{\ddagger}_{\text{enz}} = 38$ kJ mol^{−1} is equal to the difference in binding energies $\Delta G^{\text{T}}_{\text{bind}} - \Delta G^{\text{R}}_{\text{bind}}$ of the enzyme with the TS and the solvent-separated ion-pair, neither of which was evaluated in the simu-

lation. The difference $\Delta G_{\text{act}}^{\text{R}} - \Delta G_{\text{enz}}^{\ddagger} = 8 \text{ kJ mol}^{-1}$ was considered to quantify the energetic influence of the environment – either protein or water – upon the substrate as it changes from the reactant state to the transition state. However, this analysis lacks consistency in one respect, because, although the structure of $\text{S}_{\text{enz}}^{\text{R}}$ is (by definition) geometrically the same for the substrate in both the enzyme active site and in aqueous solution, the structures of S^{T} in the two different environments are not the same. A fair point of criticism for the concept of TS binding in enzyme catalysis has been that the TS need not be the same for both the catalysed and uncatalysed reactions [8]. Consequently, the previous analysis [17] should be modified by recognising that the apparent binding energy $\Delta G_{\text{bind}}^{\text{T}}$ is the sum of distortion energy $\Delta G_{\text{dist}}^{\text{T}}$ and interaction energy $\Delta G_{\text{int}}^{\text{T}}$.

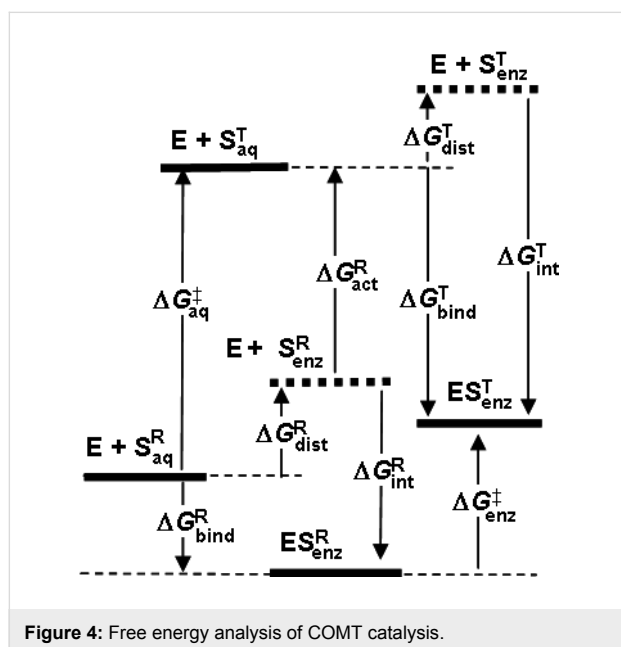
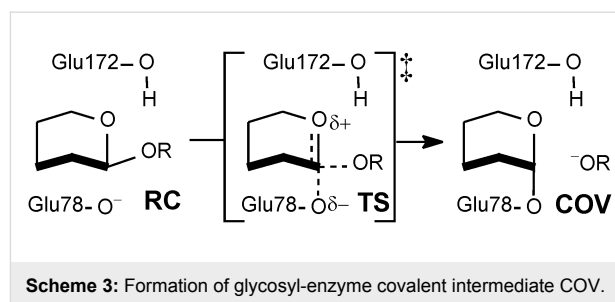


Figure 4: Free energy analysis of COMT catalysis.

The species $\text{S}_{\text{enz}}^{\text{R}}$ in water is well defined and amenable to computational investigation, although experimentally it is transient and may not necessarily correspond to a genuine intermediate. Similarly, species $\text{S}_{\text{enz}}^{\text{T}}$ in water is also well defined and amenable to computational investigation, although – unlike $\text{S}_{\text{enz}}^{\text{R}}$ in water – it was not considered in the previous work [17]. A fair evaluation of the energetic influence of the protein or water environment on the substrate as it changes from the reactant state to the transition state should be made by comparison of $\Delta G_{\text{enz}}^{\ddagger}$ with $\Delta G_{\text{act}}^{\text{R}} + \Delta G_{\text{dist}}^{\text{T}}$, since in each case the structures are the same. Owing to the structural distortions of both the reactant and transition states in going from aqueous solution into the enzyme active site, the quantity $\Delta G_{\text{bind}}^{\text{T}} - \Delta G_{\text{bind}}^{\text{R}}$ is an apparent catalytic power which differs from the intrinsic catalytic power $\Delta G_{\text{int}}^{\text{T}} - \Delta G_{\text{int}}^{\text{R}}$ by virtue of the differential distortion energy $\Delta G_{\text{dist}}^{\text{T}} - \Delta G_{\text{dist}}^{\text{R}}$.

TS recognition in enzymic glycoside hydrolysis

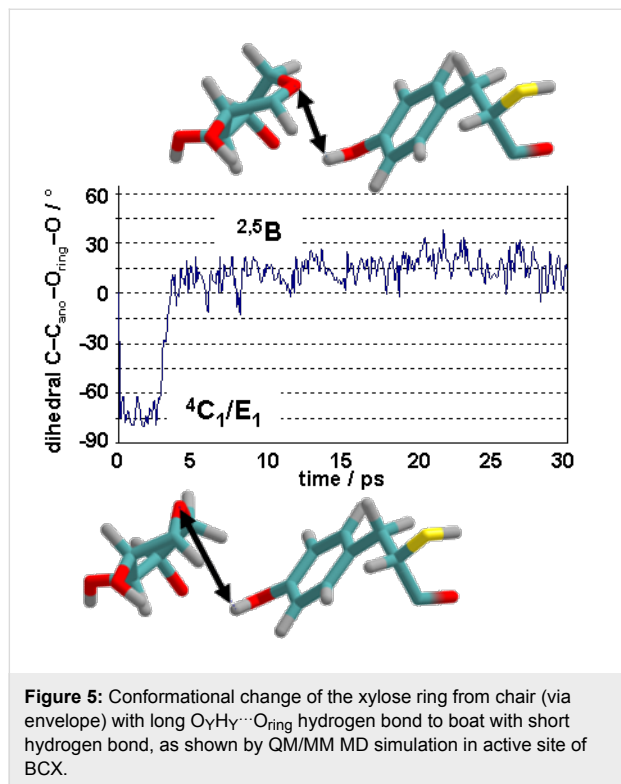
The *endo*-1,4- β -xylanase (BCX) from *Bacillus circulans* catalyses the hydrolysis of xylan and β -xylobiosides with net retention of anomeric configuration by means of a double displacement mechanism involving a covalent glycosyl-enzyme intermediate. Formation and hydrolysis of this covalent intermediate occur via oxacarbenium ion-like TSs, with the assistance of two key active site glutamic acid residues [20]. Glu78 is deprotonated in the noncovalent enzyme-substrate reactant complex: it attacks the anomeric carbon of the substrate as a nucleophile and displaces the aglycone nucleofuge (Scheme 3). Glu172 is protonated in the reactant complex and plays a dual role of acid/base catalyst: in the glycosylation step it assists formation of the glycosyl-enzyme intermediate by donating a proton to the aglycone of the natural substrate, and in the subsequent deglycosylation step it serves as a base, deprotonating the attacking water molecule. Tyr69 donates a strong hydrogen bond to the nucleophilic oxygen atom (O_{nuc}) of Glu78 in the reactant complex; in the covalent intermediate, this hydrogen bond is weaker, but a stronger interaction is formed between Tyr69 and the ring oxygen (O_{ring}) of the proximal xylose moiety of the xylobioside substrate [21]. The phenolic oxygen (O_{Y}) of Tyr69 is very important for catalysis, as evidenced by the observation that the Tyr69Phe mutant exhibits no detectable enzyme activity [22], and so it is an intriguing question to investigate the nature of this $\text{O}_{\text{Y}}\text{H}_{\text{Y}}\cdots\text{O}_{\text{ring}}$ interaction.



Scheme 3: Formation of glycosyl-enzyme covalent intermediate COV.

MD simulations with the hybrid AM1/OPLS-AA/TIP3P method showed that both ${}^4\text{C}_1$ chair and ${}^{2,5}\text{B}$ boat conformers of phenyl β -xyloside remained stable in water during the course of 30 ps trajectories, even in the presence of propionate and propionic acid moieties to mimic Glu78 and Glu172 [23]. In contrast, analogous MD simulations for the ${}^4\text{C}_1$ conformer of the reactant complex of phenyl β -xylobioside with BCX showed spontaneous transformation to the ${}^{2,5}\text{B}$ conformer (Figure 5): the conformational change is accompanied by a marked decrease in the length of the $\text{O}_{\text{Y}}\text{H}_{\text{Y}}\cdots\text{O}_{\text{ring}}$ hydrogen bond. Moreover, analogous simulations for the Tyr69Phe mutant (lacking O_{Y}) showed the chair to be stable, thereby confirming the key role of Tyr69 in preferentially stabilising the boat, with

a relative free energy difference of about 20 kJ mol^{-1} , by means of the $\text{O}_Y\text{H}_Y\cdots\text{O}_{\text{ring}}$ hydrogen bond [23].



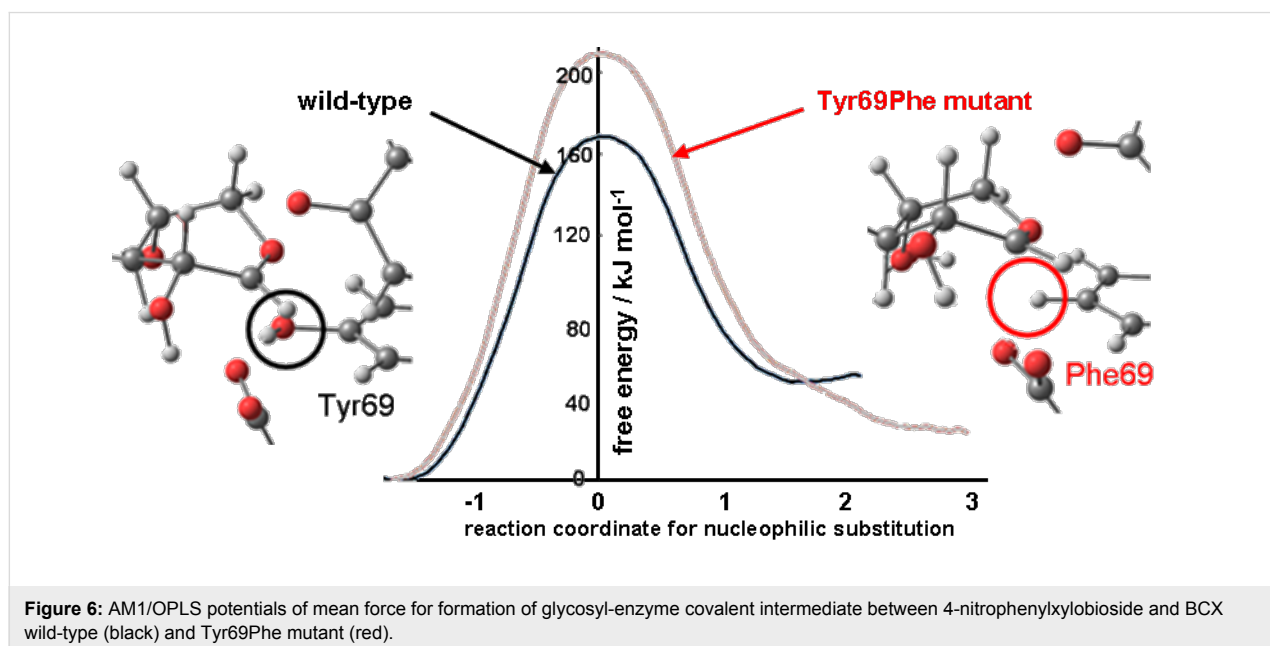
A two-dimensional PMF computed for 4-nitrophenyl β -xylobioside (the substrate employed in the experimental kinetics studies) with BCX using the same AM1/OPLS-AA hybrid potential, as a function of coordinates for nucleophilic substitu-

tion and proton transfer from Glu172, showed no requirement for protonation of the activated nucleofuge [24]. PMFs, with respect to the nucleophilic substitution reaction coordinate for both the wild-type and the Tyr69Phe mutant, computed with the same QM/MM MD method, revealed a decrease in free energy of activation of about 40 kJ mol^{-1} due to the presence of the single O_Y atom in BCX (Figure 6).

Fluctuations in the hydrogen-bond distances $\text{H}_Y\cdots\text{O}_{\text{ring}}$ (red) and $\text{H}_Y\cdots\text{O}_{\text{nuc}}$ (blue) to the boat conformer of RC, TS and glycosyl-enzyme COV intermediate in the active site of BCX, as determined by 30 ps AM1/OPLS-AA MD trajectories, are shown Figure 7. Averaged over a longer (93 ps) trajectory for RC than shown here, the mean $\text{H}_Y\cdots\text{O}_{\text{ring}}$ distance was significantly shorter ($2.47 \pm 0.49 \text{ \AA}$) than $\text{H}_Y\cdots\text{O}_{\text{nuc}}$ ($3.29 \pm 0.48 \text{ \AA}$). On the other hand, $\text{H}_Y\cdots\text{O}_{\text{nuc}}$ is consistently shorter ($1.97 \pm 0.14 \text{ \AA}$) in the TS than $\text{H}_Y\cdots\text{O}_{\text{ring}}$ ($2.39 \pm 0.20 \text{ \AA}$), indicating that the hydrogen bond between Tyr69 and Glu78 is favoured, although both distances are shorter than in RC. In COV, however, $\text{H}_Y\cdots\text{O}_{\text{nuc}}$ is once more longer than $\text{H}_Y\cdots\text{O}_{\text{ring}}$, indicating that Tyr69 now donates its hydrogen bond exclusively to the xylose ring rather than to Glu78, although the average distance to the latter is similar to that in RC. Thus it appears that stabilisation of the TS is due to the transient presence of a shorter, stronger hydrogen bond to O_{nuc} , which, of course, is absent in the TS for the Tyr69Phe mutant.

Conclusion

Catalysts work by stabilising the TS relative to reactants, but the idea of designing a “catalyst” simply to bind strongly to the TS does not always work. Selective stabilisation of the TS for



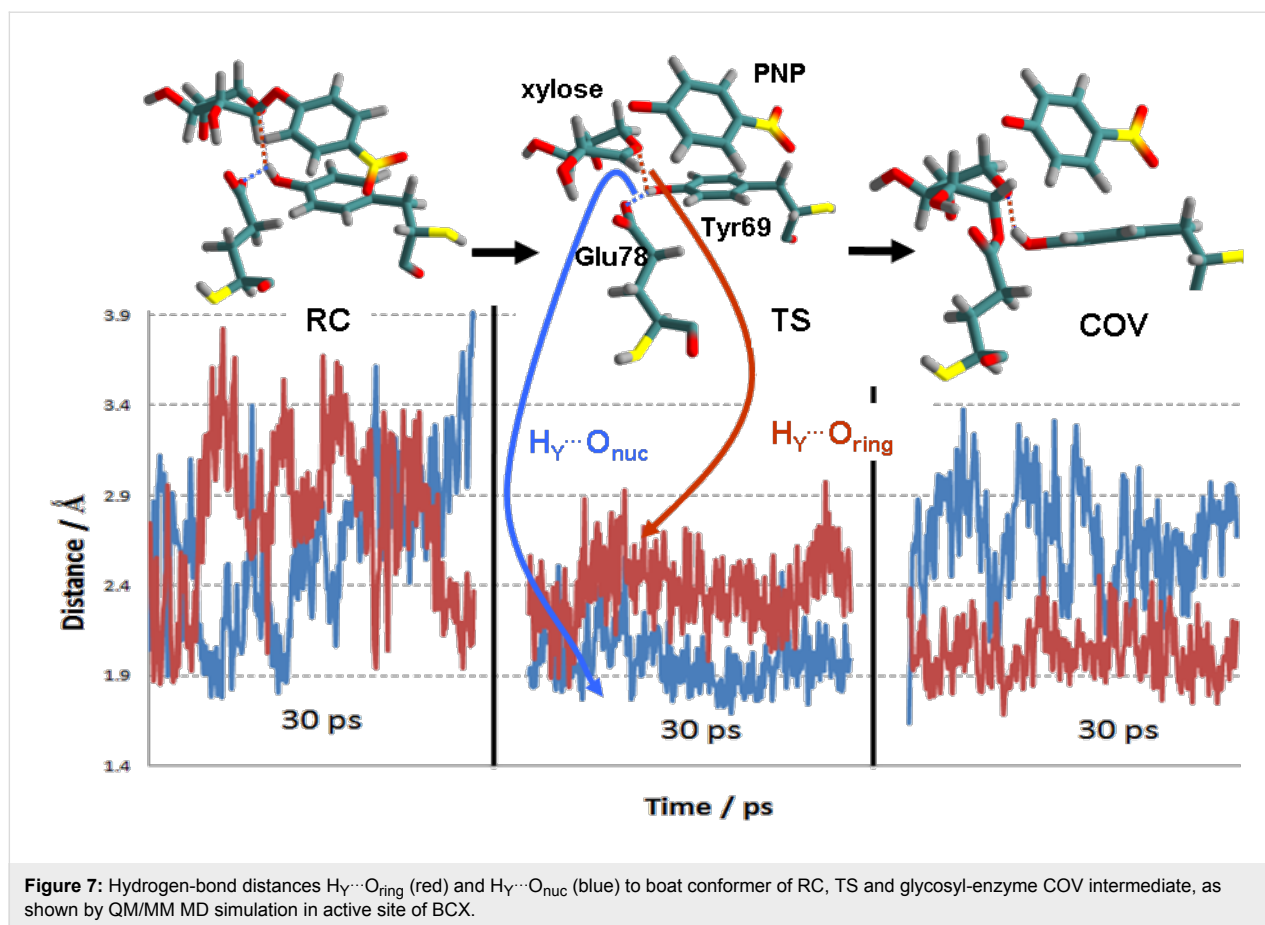


Figure 7: Hydrogen-bond distances $H_Y \cdots O_{ring}$ (red) and $H_Y \cdots O_{nuc}$ (blue) to boat conformer of RC, TS and glycosyl-enzyme COV intermediate, as shown by QM/MM MD simulation in active site of BCX.

methyl transfer could be achieved in principle by means of compression, but in practice COMT catalyses by requiring less reorganisation of the electrostatic environment to go from RC to TS than is needed in aqueous solution, thereby achieving selective stabilisation of TS. The boat conformer of a xyloside substrate is favoured over the chair in the active site of BCX owing to a hydrogen bond from Tyr69 to O_{ring} of xylose, but preferential stabilisation of the TS in the wild-type relative to a Tyr69Phe mutant is achieved by means of a short, strong hydrogen bond from Tyr69 to the enzymic nucleophile. Catalysis is TS molecular recognition, and computational simulation may provide valuable insight into the causes of preferential stabilisation.

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